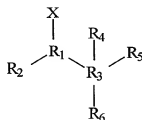


**We claim:**

1. A composition for performing a polynucleotide replication reaction, which comprises a buffer, one or more template polynucleotides, nucleotide triphosphates, one or more polymerase enzymes or fragments thereof, and one or more reaction adjuvants comprising compounds of the formula:



Formula I

wherein:

R<sub>1</sub> is C or S; and

- 15 when R<sub>1</sub> is C, X is =O, R<sub>3</sub> is N and R<sub>6</sub> is absent;

when R<sub>1</sub> is S, X is =O or  $\text{O}=\text{O}$

and R<sub>3</sub> is C;

- R<sub>2</sub> is H or CH<sub>3</sub> only when one or more of R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> is not H, and otherwise R<sub>2</sub> is an unsubstituted or halogen-, hydroxy- or alkoxy- substituted alkyl or cycloalkyl of length m, wherein m is selected such that the total number of carbons in the compound is between 3 and 8 when R<sub>1</sub> is C and between 2 and 8 when R<sub>1</sub> is S; wherein any two of R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> optionally form a cyclic structure in which cyclization is effected through a bond between them; and

- R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> each is H, alkyl, cycloalkyl or halogen-, hydroxy- or alkoxy-substituted alkyl or cycloalkyl of length n, wherein n is selected such that the total number of

carbons in the compound is between 3 and 8 when R<sub>1</sub> is C and between 2 and 8 when R<sub>1</sub> is S.

2. The composition of claim 1, wherein the reaction adjuvant comprises a  
5 cyclic compound, wherein the cyclization is effected through a bond between any two  
of R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub>.

3. The composition of claim 2, wherein the cyclic portion of the compound  
comprises five, six or seven members.

10

4. The composition of claim 3, wherein R<sub>1</sub> is C, R<sub>3</sub> is N, R<sub>6</sub> is absent and one  
of R<sub>4</sub> or R<sub>5</sub> is H.

5. The composition of claim 3, wherein R<sub>1</sub> is S and remainder of the  
15 compound is unsubstituted.

6. The composition of claim 1, wherein the reaction adjuvant comprises a  
compound in which R<sub>1</sub> is C, X is =O, R<sub>3</sub> is N and R<sub>6</sub> is absent.

20

7. The composition of claim 6, wherein the compound is cyclic.

8. The composition of claim 7, wherein the cyclic structure of the compound  
is formed by a bond between R<sub>4</sub> and R<sub>5</sub>.

9. The composition of claim 7, wherein the cyclic structure of the compound is a five, six, or seven-membered ring formed by a bond between R<sub>2</sub> and either R<sub>4</sub> or R<sub>5</sub>.

10. The composition of claim 9, wherein R<sub>4</sub> and R<sub>5</sub> are H, methyl or lower alkyl, or halogen-, hydroxy- or alkoxy-substituted lower alkyl.

11. The composition of claim 10, wherein the reaction adjuvant is selected from the group consisting of 2-pyrrolidone, N-methyl pyrrolidone and N-hydroxyethyl pyrrolidone,  $\delta$ -valerolactam,  $\epsilon$ -caprolactam and N-formyl morpholine.

10


12. The composition of claim 6, wherein the compound is acyclic.

13. The composition of claim 12, comprising a compound wherein R<sub>2</sub> is H, lower alkyl, or halogen- hydroxy- or alkoxy-substituted lower alkyl, provided that, when R<sub>2</sub> is H or methyl, one or both of R<sub>4</sub> and R<sub>5</sub> is methyl, lower alkyl, or halogen-hydroxy- or alkoxy-substituted lower alkyl.

15

14. The composition of claim 13, wherein the reaction adjuvant is selected from the group consisting of propionamide and N,N-dimethyl acetamide.

20

15. The composition of claim 1, wherein the reaction adjuvant comprises a compound in which R<sub>1</sub> is S, X is =O or , and R<sub>3</sub> is C.

16. The composition of claim 15, wherein the compound is cyclic.

17. The composition of claim 16, wherein the cyclic structure of the compound is a five, six, or seven-membered ring formed by a bond between R<sub>2</sub> and either R<sub>4</sub>, R<sub>5</sub> or R<sub>6</sub>.

5

18. The composition of claim 17, wherein the ring is unsubstituted except in R<sub>1</sub>.

19. The composition of claim 18, wherein the compound is selected from the group consisting of tetramethylene sulfone and tetramethylene sulfoxide.

10

20. The composition of claim 15, wherein the compound is acyclic.

21. The composition of claim 20, comprising a compound in which R<sub>2</sub> or R<sub>3</sub> is lower alkyl or substituted lower alkyl.

15

22. The composition of claim 21, wherein the compound is selected from the group consisting of methyl sulfone, ethyl sulfone, n-propyl sulfone, n-propyl sulfoxide and methyl *sec*-butyl sulfoxide.

20

23. The composition of claim 1, wherein the polynucleotide replication reaction is an amplification reaction.

24. The composition of claim 23, wherein the amplification reaction is selected from the group consisting of polymerase chain reaction, nucleic acid sequence-based amplification, transcription-based amplification system, self-sustained sequence replication, ligation amplification reaction, Q-beta replicase amplification and ligase  
5 chain reaction.

25. The composition of claim 1, wherein the one or more polymerases or fragments thereof is selected from the group consisting of Taq polymerase, Tth polymerase, Tme polymerase, Tli polymerase, Pfu polymerase, DNA polymerase I,  
10 Klenow fragment and reverse transcriptase.

26. The composition of claim 1, wherein the reaction adjuvant has a potency of at least 75% of the potency of DMSO or formamide in an equivalent polynucleotide chain reaction (PCR).  
15

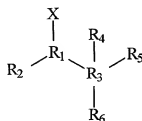
27. The composition of claim 1, wherein the reaction adjuvant has a specificity of at least 80% of the specificity of DMSO or formamide in an equivalent polynucleotide chain reaction (PCR).

28. The composition of claim 1, wherein the reaction adjuvant has an effective range spanning at least 0.1 M.  
20

29. The composition of claim 1, wherein the polynucleotide template comprises greater than 50% G+C.

30. The composition of claim 1, wherein the one or more polymerase enzymes or fragments thereof is selected from the group consisting of Taq polymerase, Tth polymerase, Tme polymerase, Tli polymerase, Pfu polymerase, DNA polymerase I, Klenow fragment and reverse transcriptase.

31. A method of performing a polynucleotide replication reaction, which comprises performing the reaction in the presence of one or more reaction adjuvants comprising compounds of the formula:



Formula I

wherein:

R<sub>1</sub> is C or S; and

when R<sub>1</sub> is C, X is =O, R<sub>3</sub> is N and R<sub>6</sub> is absent;

when R<sub>1</sub> is S, X is =O or  $\text{O}=\text{O}$

and R<sub>3</sub> is C;

- R<sub>2</sub> is H or CH<sub>3</sub> only when one or more of R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> is not H, and otherwise R<sub>2</sub> is an unsubstituted or halogen-, hydroxy- or alkoxy- substituted alkyl or cycloalkyl of length m, wherein m is selected such that the total number of carbons in the compound is between 3 and 8 when R<sub>1</sub> is C and between 2 and 8 when R<sub>1</sub> is S; wherein any two of R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> optionally form a cyclic structure in which cyclization is effected through a bond between them; and

R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> each is H, alkyl, cycloalkyl or halogen-, hydroxy- or alkoxy-substituted alkyl or cycloalkyl of length n, wherein n is selected such that the total number of carbons in the compound is between 3 and 8 when R<sub>1</sub> is C and between 2 and 8 when R<sub>1</sub> is S; and

- 5 R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> each is H, alkyl, cycloalkyl or halogen-, hydroxy- or alkoxy-substituted alkyl or cycloalkyl of length n, wherein n is selected such that the total number of carbons in the compound is between 3 and 8 when R<sub>1</sub> is C and between 2 and 8 when R<sub>1</sub> is S.

- 10 32. The method of claim 31, wherein the reaction adjuvant comprises a cyclic compound, wherein the cyclization is effected through a bond between any two of R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub>.

- 15 33. The method of claim 32, wherein the cyclic portion of the compound comprises five, six or seven members.

34. The method of claim 33, wherein R<sub>1</sub> is C, R<sub>3</sub> is N, R<sub>6</sub> is absent and one of R<sub>4</sub> or R<sub>5</sub> is methyl, ethyl or hydroxyethyl.

- 20 35. The method of claim 33, wherein R<sub>1</sub> is S and remainder of the compound is unsubstituted.

36. The method of claim 31, wherein the reaction adjuvant comprises a compound in which R<sub>1</sub> is C, X is =O, R<sub>3</sub> is N and R<sub>6</sub> is absent.

37. The method of claim 36, wherein the compound is cyclic.

38. The method of claim 37, wherein the cyclic structure of the compound is  
5 formed by a bond between R<sub>4</sub> and R<sub>5</sub>.

39. The method of claim 37, wherein the cyclic structure of the compound is a  
five, six, or seven-membered ring formed by a bond between R<sub>2</sub> and either R<sub>4</sub> or R<sub>5</sub>.

10 40. The method of claim 39, wherein R<sub>4</sub> and R<sub>5</sub> are H, methyl or lower alkyl,  
or halogen-, hydroxy- or alkoxy-substituted lower alkyl.

41. The method of claim 40, wherein the reaction adjuvant is selected from the  
group consisting of 2-pyrrolidone, N-methyl pyrrolidone and N-hydroxyethyl  
15 pyrrolidone,  $\delta$ -valerolactam,  $\epsilon$ -caprolactam and N-formyl morpholine.


42. The method of claim 36, wherein the compound is acyclic.

43. The method of claim 42, comprising a compound wherein R<sub>2</sub> is H, lower  
20 alkyl, or halogen- hydroxy- or alkoxy-substituted lower alkyl, provided that, when R<sub>2</sub>  
is H or methyl, one or both of R<sub>4</sub> and R<sub>5</sub> is methyl, lower alkyl, or halogen- hydroxy-  
or alkoxy-substituted lower alkyl.



44. The method of claim 43, wherein the reaction adjuvant is selected from the group consisting of propionamide and N,N-dimethyl acetamide.

45. The method of claim 31, wherein the reaction adjuvant comprises a

5 compound in which R<sub>1</sub> is S, X is =O or , and R<sub>3</sub> is C.

46. The method of claim 45, wherein the compound is cyclic.

10 47. The method of claim 46, wherein the cyclic structure of the compound is a five, six, or seven-membered ring formed by a bond between R<sub>2</sub> and either R<sub>4</sub>, R<sub>5</sub> or R<sub>6</sub>.

48. The method of claim 47, wherein the ring is unsubstituted except in R<sub>1</sub>.

15 49. The method of claim 48, wherein the compound is selected from the group consisting of tetramethylene sulfone and tetramethylene sulfoxide.

50. The method of claim 45, wherein the compound is acyclic.

20 51. The method of claim 50, comprising a compound in which R<sub>2</sub> or R<sub>3</sub> is lower alkyl or substituted lower alkyl.

52. The method of claim 51, wherein the compound is selected from the group consisting of methyl sulfone, ethyl sulfone, n-propyl sulfone, n-propyl sulfoxide and methyl *sec*-butyl sulfoxide.

53. The method of claim 31, wherein the polynucleotide replication reaction is an amplification reaction.

54. The method of claim 53, wherein the amplification reaction is selected from the group consisting of polymerase chain reaction, nucleic acid sequence-based amplification, transcription-based amplification system, self-sustained sequence replication, ligation amplification reaction, Q-beta replicase amplification and ligase chain reaction.

55. The method of claim 31, wherein the polynucleotide replication reaction comprises a polymerase or fragment thereof selected from the group consisting of Taq polymerase, Tth polymerase, Tmc polymerase, Tli polymerase, Pfu polymerase, DNA polymerase I, Klenow fragment and reverse transcriptase.

56. The method of claim 31, wherein the reaction adjuvant has a potency of at least 75% of the potency of DMSO or formamide in an equivalent polynucleotide chain reaction (PCR).

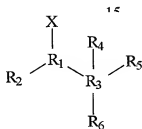
57. The method of claim 31, wherein the reaction adjuvant has a specificity of at least 80% of the specificity of DMSO or formamide in an equivalent polynucleotide chain reaction (PCR).

58. The method of claim 31, wherein the reaction adjuvant has an effective range spanning at least 0.1 M.

59. The method of claim 31, performed on a polynucleotide template comprising greater than 50% G+C.

60. A kit for performing a polynucleotide amplification reaction, comprising a container that includes:

a) one or more compounds of the formula:



Formula I

wherein:

$\text{R}_1$  is C or S; and

when  $\text{R}_1$  is C, X is =O,  $\text{R}_3$  is N and  $\text{R}_6$  is absent;

when  $\text{R}_1$  is S, X is =O or  $\text{O}=\text{O}$   
and  $\text{R}_3$  is C;

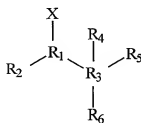
- R<sub>2</sub> is H or CH<sub>3</sub> only when one or more of R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> is not H, and otherwise R<sub>2</sub> is an unsubstituted or halogen-, hydroxy- or alkoxy- substituted alkyl or cycloalkyl of length m, wherein m is selected such that the total number of carbons in the compound is between 3 and 8 when R<sub>1</sub> is C and between 2 and 8 when R<sub>1</sub> is S; wherein any two of R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> optionally form a cyclic structure in which cyclization is effected through a bond between them; and R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> each is H, alkyl, cycloalkyl or halogen-, hydroxy- or alkoxy-substituted alkyl or cycloalkyl of length n, wherein n is selected such that the total number of carbons in the compound is between 3 and 8 when R<sub>1</sub> is C and between 2 and 8 when R<sub>1</sub> is S; and
- b) instructions for using the one or more compounds in a polynucleotide replication reaction.

61. The kit of claim 60, which further comprises one or more of:
- a polynucleotide replication reaction buffer;
  - nucleotide triphosphates;
  - oligonucleotide primers;
  - a known template polynucleotide for use as a control; and
  - one or more polymerase enzymes.
62. The kit of claim 61, customized for performing amplification reactions.
63. The kit of claim 62, customized for performing PCR.

64. A method for optimizing a polynucleotide replication reaction for a selected polynucleotide, the method comprising:

a) providing a plurality of reaction adjuvants comprising compounds of the formula:

5



Formula I

10

wherein:

R<sub>1</sub> is C or S; and

when R<sub>1</sub> is C, X is =O, R<sub>3</sub> is N and R<sub>6</sub> is absent;

when R<sub>1</sub> is S, X is =O or  $\text{O}=\text{O}$

15 and R<sub>3</sub> is C;

R<sub>2</sub> is H or CH<sub>3</sub> only when one or more of R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> is not H, and otherwise R<sub>2</sub> is an unsubstituted or halogen-, hydroxy- or alkoxy- substituted alkyl or cycloalkyl of length m, wherein m is selected such that the total number of carbons in the compound is between 3 and 8 when R<sub>1</sub> is C and between 2 and 8 when R<sub>1</sub> is S; wherein any two

20 of R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> optionally form a cyclic structure in which cyclization is effected through a bond between them; and

R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> each is H, alkyl, cycloalkyl or halogen-, hydroxy- or alkoxy-substituted alkyl or cycloalkyl of length n, wherein n is selected such that the total number of

carbons in the compound is between 3 and 8 when R<sub>1</sub> is C and between 2 and 8 when R<sub>1</sub> is S;

- b) performing a plurality of polynucleotide replication reactions on the selected polynucleotide, each reaction being performed under equivalent conditions,
- 5 but with varying type or amount of the reaction adjuvants in the reactions; and
- c) selecting the type and concentration of reaction adjuvant that yields the most favorable results for polynucleotide replication of the selected polynucleotide template, thereby optimizing the polynucleotide replication reaction for the selected polynucleotide template.

10

65. The method of claim 64, wherein the polynucleotide replication reaction is an amplification reaction.

66. The method of claim 65, wherein the amplification reaction is PCR.

15

67. A kit for performing the method of claim 64, which comprises a container in which is included:

- a) the plurality of reaction adjuvants; and
- b) instructions for using the reaction adjuvants to optimize polynucleotide
- 20 replication of a selected template polynucleotide.

68. The kit of claim 67, which further comprises one or more of:

- a) a polynucleotide replication reaction buffer;
- b) nucleotide triphosphates;

10056917-012502

- c) oligonucleotide primers;
  - d) a known template polynucleotide for use as a control;
  - e) one or more polymerase enzymes; and
  - f) one or more reaction vessels for performing the plurality of
- 5 polynucleotide replication reactions.

69. The kit of claim 68, customized for performing amplification reactions.

70. The kit of claim 69, customized for performing PCR.